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13. ABSTRACT ***(Maximum 200 Words)***

An experimental drug, PDMP, has been shown to block metastasis by inhibiting the display of carbohydrates on tumor cell surfaces. Further work with PDMP and related drugs to prevent the spread of cancer now seems justified. This work is intended to show that rapidly growing insect nerve cells can serve as a model for cancer in humans, and simple assays based on this approach should permit the testing of a large number of new drugs based on PDMP. Drugs that block the outgrowth of the insect nerve cells should also block the outgrowth of cancer cells in humans, or other vertebrate organisms. Using this approach we should be able to rapidly develop potent new drugs which will block metastasis in human cancers.

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Robin L. Poltz 25 July 2000

PI - Signature Date

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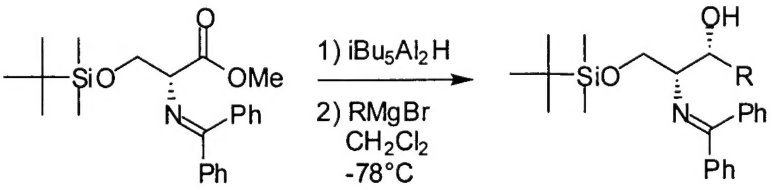
Introduction

This project consists of 4 interconnected efforts: 1) The synthesis of novel PDMP analogs, which are capable of altering GSL expression. 2) Structural determination of the GSL content in *Manduca sexta*. Ultimately, we will be able to examine the effects of the PDMP analogs on the ensemble of expressed GSL's. 3) Examination of the biological effects of the PDMP analogs on *Manduca sexta*. Ultimately, this will involve studies performed at the cellular level (cell culture experiments), tissue level (neuronal pathfinding experiments), and at the behavioral level (mating and feeding behaviors). 4) Examination of the biological effects of the PDMP analogs on human cancer cell invasiveness. These results will be compared with results from the *Manduca* experiments. The first two parts of the project are being performed in the Polt Group laboratory in the Chemistry Department at the U. of A. The 3rd part is being performed in the Hildebrand Group laboratory in the Neuroscience Department at the U. of A. The 4th part is being performed in the McGovern Group laboratory in the Arizona Cancer Center at the University Medical Center. What is key to the collaboration is the fact that biological results are used to re-design the drugs, so that more effective anti-metastasis compounds can be developed. Hopefully, the overall results of these 4 studies will lead to useful structure-activity relationships for the GSL's themselves, in addition to the PDMP inhibitors used to perturb GSL expression *in vivo*. Similar efforts using mammalian systems have been unsuccessful due to technical difficulties, as well as the diverse immunological effects of GSL's. Insects, which lack a cellular immune system should show the cell-modulatory effects of GSL's, uncomplicated by immunological responses.

Progress in the Synthesis of *threo*-PDMP Analogs

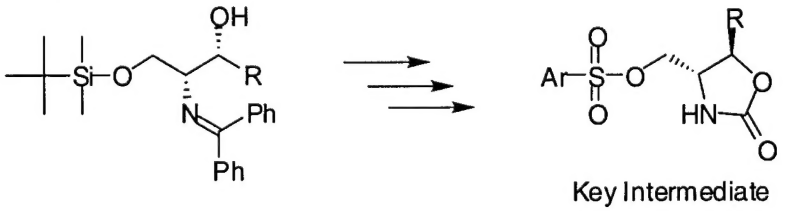
Solution-Phase Synthetic Methodology. The Polt Group has significant manpower devoted to this research work: 1st year graduate students Peter J. "Jake" Slavish and Mark R. Lefever, and 3rd year graduate student Michael M. Palian. An important observation made is that *aliphatic* Grignard reagents undergo stereoselective (>20:1) addition to Schiff base esters of serine. Thus, the reductive alkylation methodology that forms the core of our synthetic approach to glycosyltransferase inhibitors is not limited to the addition of aromatic and olefinic nucleophiles. A preliminary report of Mike's work has been submitted for publication.[1]



R	Yield	d.r.	
CH_3	60%	>20:1	**2a**
C_6H_{13}	60%	>20:1	**2b**
$C_{10}H_{21}$	58%	>20:1	**2c**

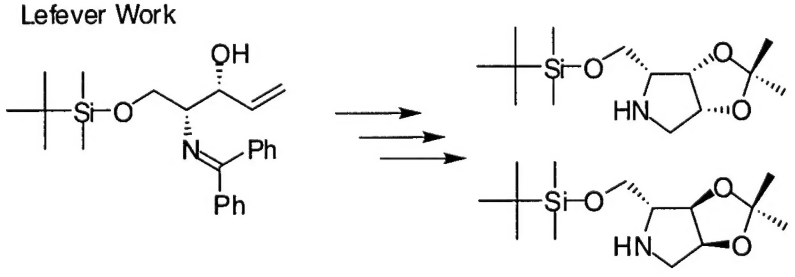
The 1st year students have made remarkable progress over the course of the summer. Jake has begun the synthesis of the key intermediate used to produce PDMP analogs, and Mark has begun the synthesis of two 5-carbon aza-sugars for attachment in both solution and solid-phase work.

Slavish Work



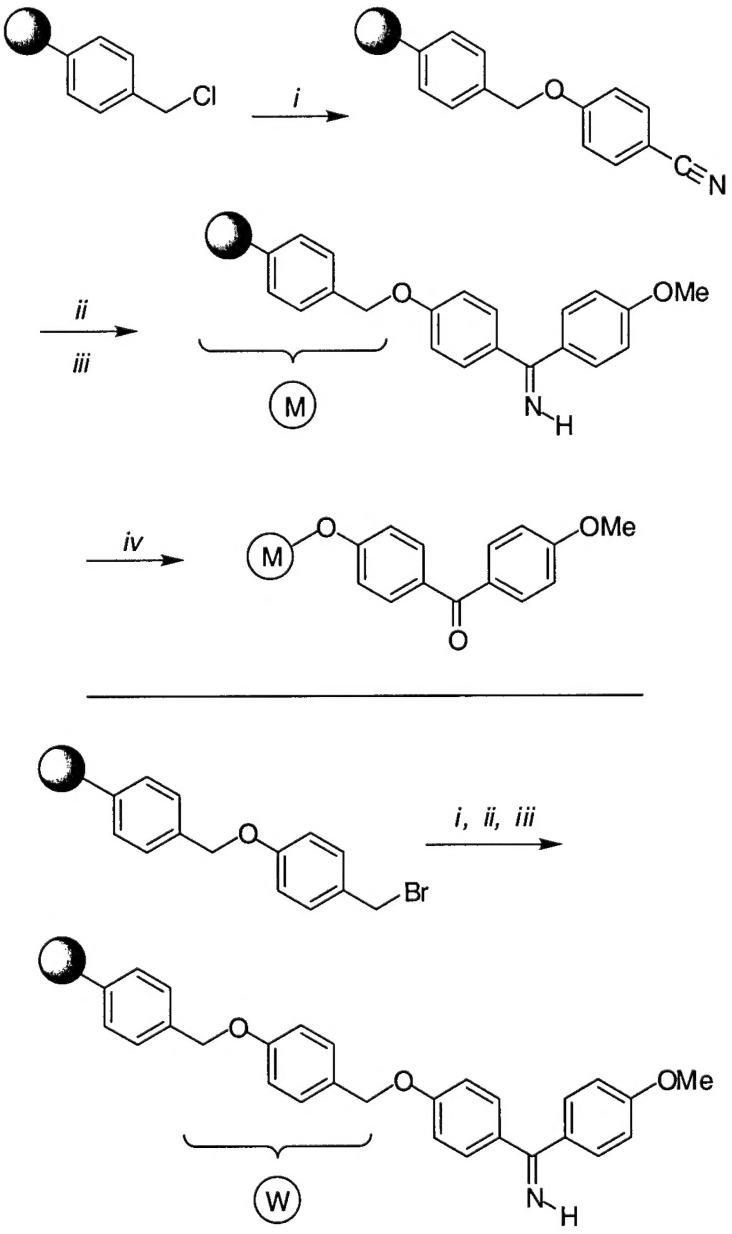
Key Intermediate

Lefever Work



Solid Phase Organic Chemistry (SPOC). Significant resources have been invested in the development of solid-phase variants of the above chemistry, with post-doctoral associate Dr. Bennett H. Novak (Ph.D., University of Florida, Gainesville, FL) and 5th year graduate student Brian D. Dangel. A preliminary report of this work has been submitted for publication.[2]

Merrifield and Wang Attachments (See Ref. 2)



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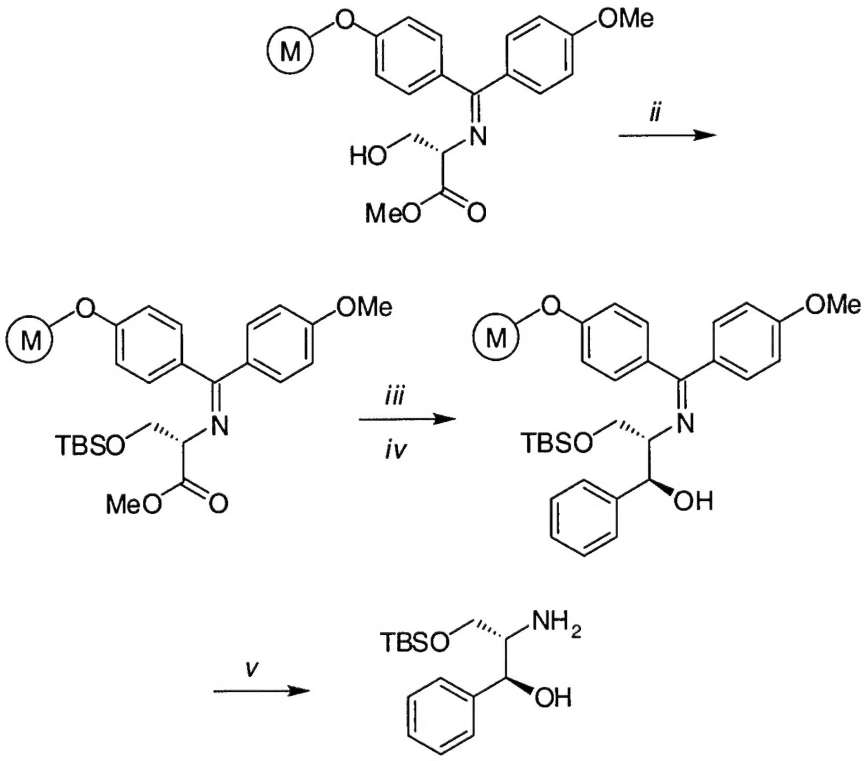


Isolation of Glycosphingolipids from *Manduca sexta*

This work was performed in the Polt laboratory by 1st-year graduate student James J. Glick, and undergraduates Beth A. Melnik and Will H. Taylor

Extraction Protocols. In an effort to determine the optimal extraction protocol 5 complete extractions have been performed consuming about 60 larva (~10 g each). The extractions were performed on 5th instar *Manduca sexta* using the various modifications of the Folch procedure.[3] Modifications included "wet" extraction of homogenized larva, lyophilization prior to extraction, removal of the gut prior to homogenation to remove GSL contributions from plant sources (*e.g.* wheat GSL's from "bug chow"), and different M.W. cut-offs for dialysis of the extracts. Typically, 10 larva provide a mixture of 65—76 mg of dry GSL's for an overall extraction yield of 0.06—0.09% on a dry weight basis.

Mass Spectrometry Data – Folch Lipid Fraction. The chloroform layer of the Folch extraction that contained the lipids was rotary evaporated and the lipid converted to the methyl ester and analyzed by GC-MS with an electron impact (EI) detector. **Table 1** lists the percent composition of the derivatized lipids based on the GC-MS (EI) data:

Table 1. Normalized Percent Composition of Lipid Components in Chloroform Layer

	WHT-I-83b	JJG-I-15	JJG-I-15†
C_{12}	0.000	0.000	0.000
C_{14}	0.000	0.000	0.000
C_{16} =	1.800	1.700	1.938
C_{16}	33.700	25.700	29.298
C_{18} = =	32.100	24.800	28.272
C_{18} =	26.300	27.600	31.464
C_{18} =	4.700	4.700	5.358
C_{20}	0.000	0.400	0.456
C_{20} = = = =	0.000	12.500	0.000
Unk.	1.200	2.800	3.192
Total %	99.8	100.2	100.0

✱ Observed below the threshold limit of detection

= Represents the presence of a double bond(s)

† Percentages w/omission of arachadonic acid

The presence of the C_{16} through C_{18} fatty acids in WHT-1-83b, in addition to unsaturated C_{15} and unsaturated C_{19} fatty acids (*yes, an odd number of carbons!*), was confirmed using negative FAB on underivatized fatty acids. The presence of a suspected C_{20} fatty acid with degrees of unsaturation in JJG-1-15, which solidified during rotary evaporation, was identified by an infrared library match to be arachidonic acid. This was not observed in the first extraction, since the fatty acid fraction was removed in that case by trituration with acetone. A variety of ionization techniques have been employed to elucidate the structures of the isolated materials. Presently, there does not appear to be a single ionization technique that provides information on all the compounds believed to be in the isolate.

Electrospray ionization (ESI) in the positive mode of extracts collected by Beth, for example, yielded several strong peaks with mass to charge ratios (m/z) of 712.3, 730.3, and 785.2 (BAM-1-15b) which are believed to be singly charged monosaccharide GSLs with various chain lengths. ESI in the negative mode yielded a strong ion at m/z 787 as well as a weaker ion at m/z 700 (BAM-1-15c) as the most abundant ions. Fast Atom Bombardment, which is typically used for the analysis of GSLs and carbohydrates, was also run on Beth's extract in the positive mode. Peaks at m/z 730.8 and 785.8 were observed but not the peak and m/z 712. The lack of similar peak masses using the different ionization techniques highlights the need for multiple ionization techniques to get the maximum amount of information. **Table 2** summarized the data collected on all the samples isolated to date.

Table 2. Summary of Major Masses Observed in Methanol Layer of Folch Extracts

Sample ID	Ionization Technique	Major Masses Observed (m/z)
BAM-1-15	+ FAB	519, 600, 730, 785
BAM-1-15	+ ESI	514, 702, 724, 752, 807, 1042
BAM-1-15b	+ ESI	712, 730, 785
BAM-1-15c	- ESI	600, 700, 787, 862,924
JJG-1-15	- FAB	409
JJG-1-17	- FAB	409, 705, 766
WHT-1-55b	+ ESI	516, 754, 778, 780
WHT-1-55c	+ FAB	550, 574, 600, 781
WHT-1-83a	+ FAB	336, 412, 477, 519, 557, 602, 639, 702, 730, 757, 785
WHT-1-83a	- FAB	320, 342, 411, 460, 477, 611, 640, 674
WHT-1-83b	- FAB	601, 618, 646, 689, 717, 744, 772, 824, 861, 912, 938
WHT-1-83a	MALDI	519, 576, 600, 868, 894, 918
WHT-1-109b	- FAB	409, 705, 766
WHT-1-139	+ ESI	628, 688, 724, 950, 1102

GSL Pharmacology in Cultured *Manduca sexta* Cells

This work has been performed in the Hildebrand laboratory by undergraduate biology student Christopher J. Biland and staff scientist Dr. Lynne A. Oland.

During the current funding period, we carried out a series of observations in culture on the effect of D- and L-PDMP on the growth of explanted olfactory receptor neurons and dissociated antennal-lobe (AL) neurons. Explants were made by short exposure to collagenase/dispase followed by coarse trituration of antennal epithelium from stage 4 (of 18 metamorphic stages) animals, when axons have only recently been extended. The AL neurons were dissociated according to our standard protocol (Hayashi and Hildebrand, 1990). Dose-response series were carried out for each agent, with control cultures exposed only to the aqueous DMSO vehicle.

Axons from olefactory receptor neurons (ORN) in control cultures extend radially from the explanted epithelium (**Fig. 1A**) with some axon fasciculation (visible as thick bundles), small growth cones on the tips of some axons, and most of the growth completed by 3 divisions. Explants exposed to 1 μM D-PDMP showed the same pattern, but at 5 μM or higher, many fewer explants showed outgrowth, and the outgrowth was less robust (**Fig. 1B**), possibly with less fasciculation. Cells within the explant appeared more rounded than usual. At concentrations >10 μM (**Fig. 1C**), few explants remained adherent to the substrate. This pattern has been reported previously in mice,[4] and in human leukemia cells,[5] suggesting that the olfactory axons in the moth may depend upon GSLs for adherence either to each other or to cells in the environment through which they are navigating toward their targets.[6] Axon outgrowth also may be somewhat slower, but this observation must be verified. No differences from control were seen after exposure to 1-10 μM L-PDMP.

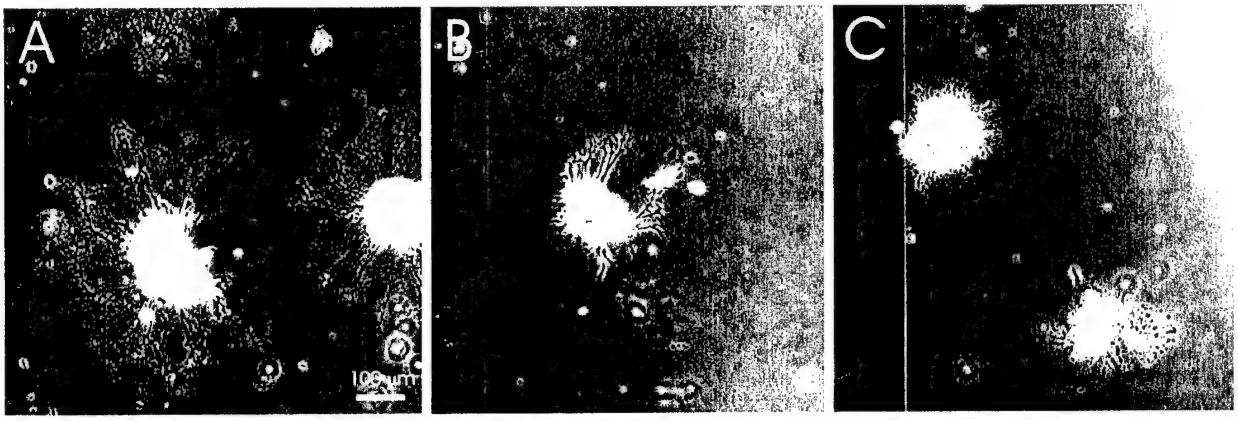


Fig. 1. Antennal explants in DMSO control (A) and D-PDMP-treated cultures (B: 1.0 μM; C: 10.0 μM). Olfactory receptor axon outgrowth in the treated explants was less robust and less extensive.

A somewhat different growth effect was seen when AL neurons were exposed to either D- or L-PDMP. The AL neurons were grown in culture for up to 2 weeks in the presence of either D- or L-PDMP for the entire culture period. The culture medium with the appropriate drug dosage was partially replaced every 3-4 days. Control cultures were exposed only to the aqueous DMSO vehicle. Quantification of outgrowth was not carried out for these initial experiments. Cultures were examined qualitatively for amount of branching, neurite length, neurite robustness, size of growth cones, and neuron survival. In addition in some experiments all examples of identified neuronal types[7] appearing at each dosage tested were photographed and compared with respect to these same features.

Neurons exposed to 5 μM D-PDMP or higher tended to appear spindly, with less branching, but there was considerable variation in the effect, with the neurons in some experiments showing little, if any, effect of exposure. We have modified the protocol by which the drugs are placed in the medium and currently are repeating the dosage series. Experiments using L-PDMP have only recently been initiated, but initial results are quite intriguing. Neurons exposed to L-PDMP may have growth cones whose morphology and frequency varies from that seen in control cultures, suggesting either a change in the frequency at which the growth cones pause and become complex, or a change in adhesion to the substrate. We have not yet examined the known cell types to determine whether they show changes in length or branching, both effects that would be expected if growth cone behavior changes.

In addition, we began to test the effect of direct injection of D-PDMP in animals at early stages of metamorphic adult development, when most of the animal is undergoing reorganization to subserve flight and reproductive requirements rather than growth. In particular, the nervous system undergoes massive reorganization, with some neurons dying, some retracting and extending processes, and some newly generated. During this period, sensory structures in the periphery extend processes to the central nervous system (CNS), and thus depend on a variety of molecular cues for guidance to their targets and formation of synaptic connections. Motor pathways to the periphery also are being established, with similar requirements. Circuitry among neurons intrinsic to the CNS also are being rewired. The goal of drug injection is to determine whether, in vivo, exposure to the drug alters the formation of peripheral nerves, guidance to the CNS, development of neuron shape, or synaptogenesis.

Our first injections of 30 mm D-PDMP were made through the wing cuticle, a route that depends on hemolymph circulation for drug dispersal and that has been used successfully for the injection of other agents, for example, hydroxyurea. These animals showed no change in their rate of development through metamorphosis, nor any changes in their gross brain

anatomy. Sections of the brain viewed at the light microscopic level showed no major changes in overall brain architecture. Anti-fasciclin II labeling, which showed the distribution of olfactory axons in the nerve and in a subset of glomeruli within the AL (**Fig. 2B**), and propidium iodide staining (nucleic acid stain), which showed a normal distribution of cell bodies (**Fig. 2C**). In some animals, unusual dilations of the head cuticle at the junctures between cuticular plates did appear, but were not extensive. However, several potential problems need to be addressed before concluding that there are no morphological effects: ❶ Drug solubility in the hemolymph may be different from that in standard culture medium; ❷ Adequate drug delivery to the head may require injection directly into the head (this is the case for another drug in use in the lab that shares with PDMP relatively low water solubility); ❸ The half-life of the drug in the animal may be too short and require repeated injections to sustain adequate levels for the animal to progress to different stages; ❹ The dosage may simply be too low. Each of these potential problems can be tested readily.

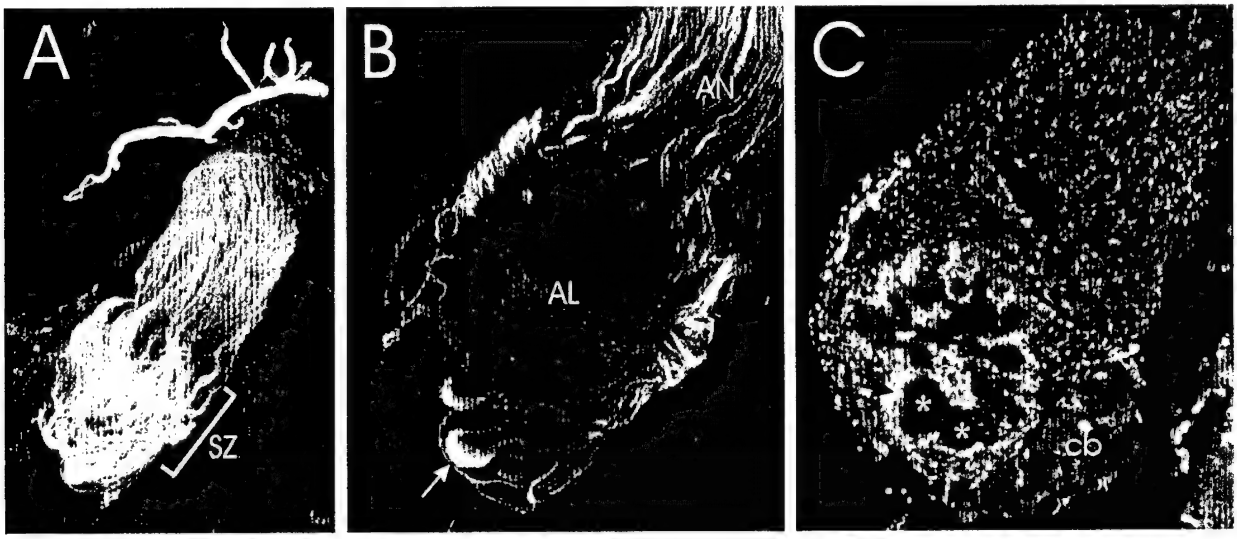


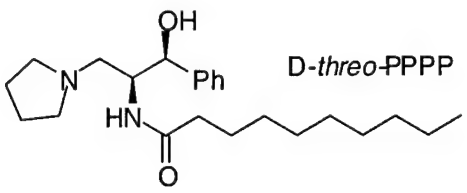
Fig. 2. Organization of antennal lobe (AL) exposed to D-PDMP at stage 5 and removed at stage 7. **A, B**: Pattern of anti-fasciclin II labeling in the olfactory axons in the antennal nerve (AN), in the sorting zone[8] (SZ) of the nerve, and in the glomeruli of the AL was normal. One glomerulus strongly positive for fasciclin-II-positive axons is indicated with an arrow. **C**: Propidium iodide staining of cell bodies. Brightly stained, small cell bodies are the glial cells of the nerve, entering from the right, and the AL. The distribution of glial cells surrounding the developing glomeruli (asterisks) and in the nerve is normal. Cell bodies (cb) of antennal lobe neurons.

Based on all of these studies, we will examine the effects of D-PPMP at the 2 μM, 10 μM and 20 μM, which showed clear-cut differences in effects. The most effective drug of those studied (D-PDMP, D-PPMP, D-PPPP, L-PDMP, L-PPMP and L-PPPP) was L-PPPP by far. Clearly, this warrants further study, since the L-series are not believed to interact with the glucosyl transferase, but show increased GSL levels in mammalian systems.[9]

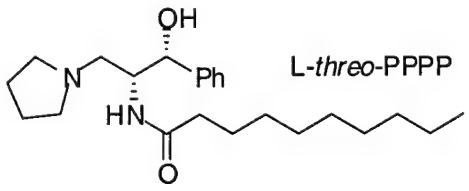
Effects on Cultured Human Breast Cancer Cells

This work, performed in the laboratory of Asst. Prof. Kathy Ann McGovern, examines the effects of the PDMP analogs on the invasiveness of human breast cells through a basement membrane model (Matrigel®) in a cell invasion chamber. In this widely-accepted[10] tumor cell invasion assay highly metastatic cancer cells are placed on a semi-permeable membrane (8-12 μm pores) with incomplete media. On the other side of the membrane is complete media, which motivates the cells to migrate toward the growth factors in the media at a certain rate. After a certain period of time the number of cells that have migrated through the Matrigel® barrier can be counted. Thus, migratory rates for a particular cell lines can be established in this way, and the effects of drugs on those invasion rates can be quantified. We hope to correlate our assay results with *Manduca sexta* nerve cells with this well-established tumor cell invasion assay.

The migratory rates for highly invasive cell line HT-1080 has been established to be 37±7% after 22 hrs at 37°C. It was found that two of our PDMP analogs were completely effective in blocking migration, while 6 others were found to be only marginally effective. Interestingly, the two compounds most effective were enantiomers.



D-*threo*-PPPP



L-*threo*-PPPP

Key Research Accomplishments

- Clearly observable effects of GSL manipulation by the PDMP compounds were seen in *Manduca sexta* cell cultures.
- L-PPPP has the most *inhibitory* potent effects on neurite extension of *M. sexta* explants.
- The effects of GSL manipulation by both D- and L-PPPP were detected in tumor cell invasion assays, using human breast cells. This is remarkable, since L-PPPP is not believed to be an inhibitor of glycosylceramide synthase.
- Reductive alkylation can be performed with saturated as well as unsaturated carbon nucleophiles.
- Analytical techniques (such as TLC and GC-MS) have been developed in the Polt laboratory to provide screening information on the extracts.
- The presence of unsaturated odd-carbon number fatty acids (C_{15} and C_{19}) in the chloroform layer of the Folch extracts has been confirmed by Fast Atom Bombardment (FAB) in two different extracts at levels approaching 1 wt%.

Reportable Outcomes

1) Palian, M.M.; Polt, R. "Short, Sterospecific Syntheses of β-Hydroxy Lipo-Amino Acids and Their Glycosides" *Organic Letters* submited.
2) Dangel, B.D.; Polt, R. "Polymeric Supports for Solid-Phase Organic Synthesis: β-Amino Alcohols, Aziridines and Transition Metal Complexes" *Angew. Chem. Int. Ed. Engl.* submitted.
3) Dangel, Brian D.; Polt, Robin. *New polymeric support for solid-phase organic synthesis: Formation and cleavage of aziridines and transition-metal complexes derived from O'Donnell's Schiff bases.* Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000 (2000)
4) Polt, Robin L.. Benzophenone schiff bases of α-amino acid esters as electrophiles. Addition of grignard reagents and alkyllithiums to produce *threo*-amino alcohols and amino polyols. *Amino Acid Derivatives. A Practical Approach,* p.p. 101—114, G.C. Barrett, (Ed.) Oxford University Press, Oxford. 101-114 (1999), .

Conclusions

- Biological activity has been observed at the cellular level in two distinct assays (*Manduca sexta* nerve cells and in HT-1080 cells). This suggests that insect cell assays may correlate with anti-metastasis activity, and that this activity may be mediated by GSL expression in these two very different species. Clearly, further study of these glycosyltransferase inhibitors is warranted. *Manduca sexta* explants will be studied at 2 μM, 10 μM and 20 μM concentrations of D-PPMP, D-PPPP and L-PPPP to establish baseline effects on the inhibition of neurite extension.

References.

[1] Palian, M.M.; Polt, R. "Short, Sterospecific Syntheses of β-Hydroxy Lipo-Amino Acids and Their Glycosides" *Organic Letters* submited.

[2] Dangel, B.D.; Polt, R. "Polymeric Supports for Solid-Phase Organic Synthesis: β-Amino Alcohols, Aziridines and Transition Metal Complexes" *Angew. Chem. Int. Ed. Engl.* submitted.

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[4] a) GM_3-enriched microdomain involved in cell adhesion and signal transduction through carbohydrate-carbohydrate interaction in mouse melanoma B16 cells. Iwabuchi, K.; Yamamura, S.; Prinetti, A.; Handa, K.; Hakomori, S. *J. Biol. Chem.* **1998**, *273,* 9130—8. b) New directions in cancer therapy based on aberrant expression of glycosphingolipids: anti-adhesion and ortho-signaling therapy. Hakomori, S. *Cancer Cells* **1991**, *3,* 461—70.

[5] A synthetic ceramide analog, D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol, selectively inhibits adherence during macrophage differentiation of human leukemia cells, Kan, C-C.; Kolesnick, R.N. *J. Biol. Chem.* **1992**, *267,* 9663-67.

[6] There is Langmuir-Blodgett evidence that GSL-containing bilayers can interact directly with other bilayers containing GSL's. For example, see: Molecular forces between membranes displaying neutral glycosphingolipids - evidence for carbohydrate attraction. Yu, Z.W.; Calvert, T.L.; Leckband, D. *Biochemistry* **1998** *37,* 1540-1550.

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[8] Rossler, Wolfgang; Oland, Lynne A.; Higgins, Mark R.; Hildebrand, John G.; Tolbert, Leslie P. Development of a glia-rich axon-sorting zone in the olfactory pathway of the moth *Manduca sexta. J. Neurosci.* **1999**, *19,* 9865-9877.

[9] See Refs. 11 and 12 in Mitchell, et al. *J. Org. Chem.* **1998**, *63,* 8837-42.

[10] a) Liotta, L.A., *et al. Cancer Metastasis Rev.* **1982**, *1,* 277, b) Hendrix, H.J., *et al. Cancer Letters* **1987**, *38,* 137, c) Repesh, L.A. *Invasion Metastasis* **1989**, *9,* 192.

Short, Stereospecific Syntheses of β-Hydroxy Lipo-Amino Acids and Their Glycosides

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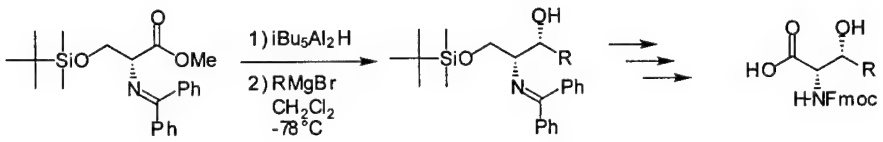
ABSTRACT



A concise, stereoselective synthesis of Fmoc β-hydroxy alkyl amino acids (or lipoamino acids) is described. iBu_5Al_2H-RMgX-induced reductive alkylation produced the desired *threo* products with greater than 20:1 diastereoselectivity. After facile protecting group exchange and cleavage, the primary alcohols were selectively oxidized directly to the carboxylic acid in the presence of unprotected secondary alcohols, or in the presence of the secondary glycoside. These amino acids and glycosides are useful in solid-phase peptide and glycopeptide synthesis.

β-hydroxy amino acids, including threonine, serine and other more unusual amino acids, are an important class of chiral, bioactive molecules.[1] In addition to being constituents of bioactive peptides such as the cyclosporins,[2] these amino acids are also components of various natural products, including vancomycin and bouvardin. Amino acids are also quite versatile synthetic intermediates for other functionalities[3], such as β-lactams[4], β-fluoro amino acids[5], and aziridines.[6]

The presence of β-hydroxy amino acids within a peptide allows for even greater synthetic versatility. More specifically, the hydroxylated side chain allows the peptide to be glycosylated.[7] This is critical for a number of reasons, among which are molecular recognition,[8] stability to enzymatic degradation[9] and enhanced transport and bioactivity.[10] New methods for the synthesis of enantiomerically pure natural and unusual amino acids are of continuing interest.

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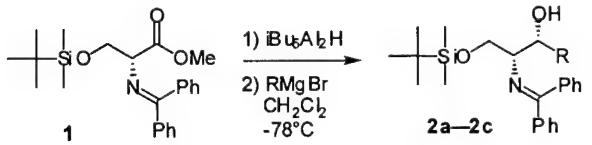
A number of lipo-amino acid syntheses have been designed,[11] but few are amenable to large-scale synthesis or possess a side chain capable of being glycosylated. Herein, we wish to report a concise, stereospecific synthesis of β-hydroxy Fmoc amino acids and other derivatives using methodology developed in this laboratory.[12,13]

In all experiments, "fully-protected" serine[12] was used as starting material. The methyl ester was reductively alkylated,[14] using the Schiff base as the source of chirality. The side chain hydroxyl was protected as the silyl ether, and remained intact throughout the sequence. In these experiments, alkyl Grignard reagents were used to produce the longer-chain analogs of threonine. No isobutyl transfer from DIBAL to the product was observed, which was unexpected considering the putative mechanism of this reaction.[12] Nonetheless, regardless of chain length, all reductive alkylations proceeded in moderate yield (~60%) with excellent diastereoselectivity for the *threo* product.[15] The products of these reactions were then converted to their respective β-hydroxy amino acids.

Scheme 1: *Reductive Alkylation of Serine Schiff Bases*

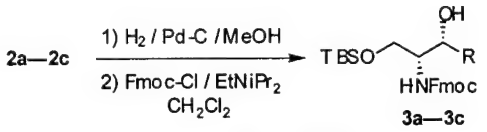


R	Yield	d.r.	
CH_3	60%	>20:1	2a
C_6H_{13}	60%	>20:1	2b
$C_{10}H_{21}$	58%	>20:1	2c

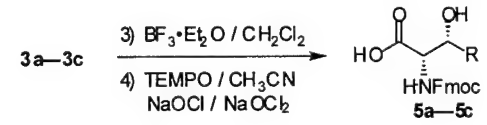
Cleavage of the Schiff base was effected by hydrogenation, followed by reprotection as the Fmoc carbamate, without purification of the intermediate free amino alcohol. This proceeded in 75-82% yield over two-steps. Lewis acidic cleavage of the silyl ether was then carried out using BF_3•OEt_2, in overall good yields (78-92%). The use of BF_3•OEt_2 was a suitable alternative to standard methods of silyl cleavage, as aqueous HF gave poor yields and TBAF resulted in quantitative loss of the Fmoc protecting group.

In the final step the Fmoc diol was subjected to chemoselective oxidation of the primary alcohol to the carboxylic acid in the presence of the unprotected secondary alcohol. This was accomplished using a TEMPO oxidation procedure[15c,16] refined by a group at Merck.[17] Although the substrate examined at Merck contained solitary hydroxyl groups, we observed no over-oxidized (di-keto) products with the diols studied. With increasing alkyl chain length, it was observed that the oxidations proceeded more slowly, which is probably a result of steric interactions at the side chain. Poor yields in the oxidation of decyl Fmoc diol are a direct result of these interactions. The final step of this sequence proceeded in 14-96% yield. This provided the methyl, hexyl and decyl β-hydroxy lipo amino acids in respectable overall yield, in seven steps from D-serine methyl ester.

Scheme 2: *Synthesis of β-Hydroxy Amino Acids*



R	Yield	Prod.
CH_3	75%	3a
C_6H_{13}	82%	3b
$C_{10}H_{21}$	81%	3c



R	Yield	Prod.	Yield	Rxn Time	Prod.
CH_3	80%	4a	96%	12 hr	5a
C_6H_{13}	92%	4b	94%	24 hr	5b
$C_{10}H_{21}$	78%	4c	*14%	>60 hr	5c

* 80% recovered starting material

A shorter, more concise sequence was attempted for the synthesis of L-*threo*-2-amino-3-hydroxy-tridecanoic acid. The product of reductive alkylation, using decyl magnesium bromide, was subjected to 2M HCl in dioxane. This served to simultaneously cleave the Schiff base imine and silyl ether, providing the 13-carbon amino diol. Without purification, the reaction mixture was made basic with solid $NaHCO_3$ and reprotected as the carbamate, using Fmoc-Cl under Schotten-Baumann conditions.[18] This provided the

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[14] In the hexyl and decyl cases, the ethyl ester was also used, and produced no increase in yield compared to the methyl case.

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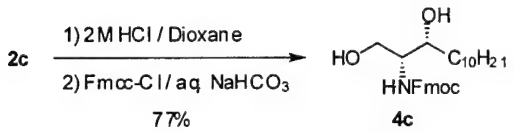
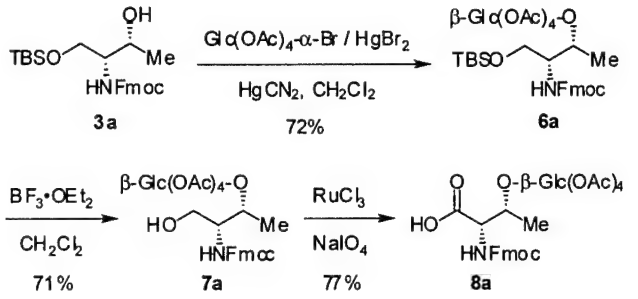
decyl Fmoc diol in 77% yield over three transformations. The diol was then oxidized to the carboxylic acid as described above. This produced the desired β-hydroxy amino acid in 6 steps from D-serine methyl ester.

Scheme 3: *Synthesis of Decyl Fmoc-AminoDiol*



In addition, these amino acid derivatives provide the opportunity for glycosylation, to yield a "sphinga-glycosyl amino acid." In preparation for glycosylation, the product of reductive alkylation using methyl Grignard was hydrogenated and reprotected as the Fmoc carbamate, as shown in **Scheme 1**. The Fmoc silyl alcohol was then glycosylated using Helferich conditions,[19] (**Scheme 4**) to give the glycosyl adduct in 72% yield with greater than 20:1 selectivity for the β-product shown. The conditions used in this case were superior to standard Keonigs-Knorr methods. Following the procedure above, the silyl ether was removed in 71% yield and the primary alcohol oxidized to the acid. However, using the TEMPO procedure this oxidation was much more sluggish than those previously discussed. Again, this is probably due to sterics. Using TEMPO, the final oxidation proceeded in an unoptimized yield of 43%. Since there is no secondary alcohol in this case, another less selective method of oxidation was employed. Oxidation with $RuCl_3/NaIO_4$ gave the Fmoc methyl β-glycosyl amino acid in a 77% in the final oxidation.

Scheme 4: *β-Glycosyl Fmoc-Threonine*



A short, stereospecific route to *threo* β-hydroxy lipo-amino acids and glycosyl lipo-amino acids, amiable to large-scale synthesis, has been presented. This route appears to be the method of choice for the oxidation of the glycosyl amino alcohols of this type, and lays the groundwork for the preparation of lipophilic peptide moieties. These compounds will provide routes to *threo* sphinga-glycosyl acids to be used to the solid-phase peptide synthesis of sphinga-glycopeptides. These compounds may possess interesting drug transport activity[10,20] since they are amphiphilic peptides, possessing a hydrophilic carbohydrate and lipophilic alkyl chain.

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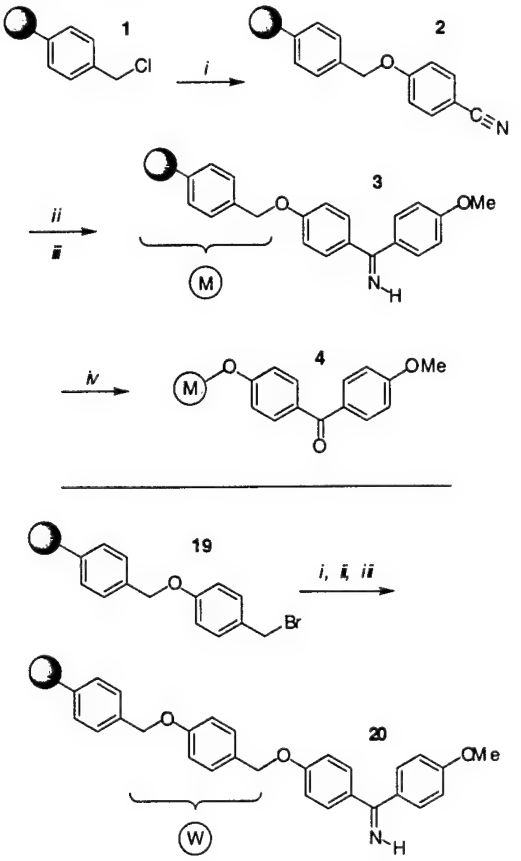
Polymeric Supports for Solid-Phase Organic Synthesis: β-Amino-Alcohols, Aziridines and Transition Metal Complexes.**

Brian D. Dangel and Robin Polt*

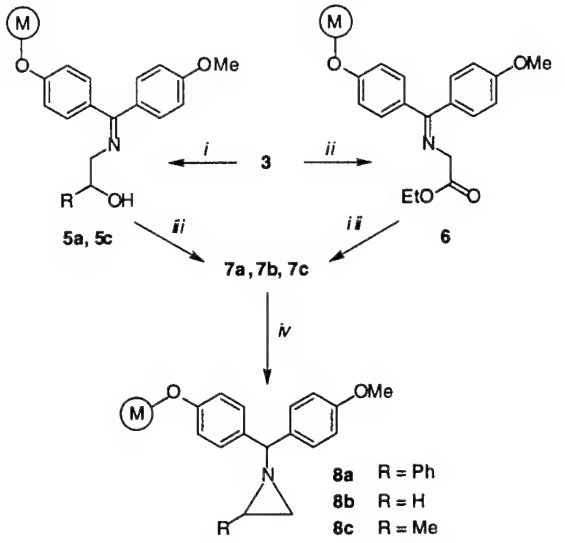
Solid phase organic chemistry (SPOC) has found numerous applications,[1] and has been used to facilitate catalyst development in both academia and industry.[2] Although Schiff bases are sometimes regarded as unstable intermediates, and are generally subjected immediately to further reactions, O'Donnell, *et al.* have demonstrated that sterically hindered benzophenone imine Schiff bases can serve as stable and extremely versatile protecting groups for primary amines.[3]



Scheme 1. Reagents: *i.* p-HOPhCN / Cs_2CO_3 / DMF / 60°C, *ii.* *p*-MeOPhMgBr / THF / 60°C, *iii.* MeOH, *iv.* H_3O^+

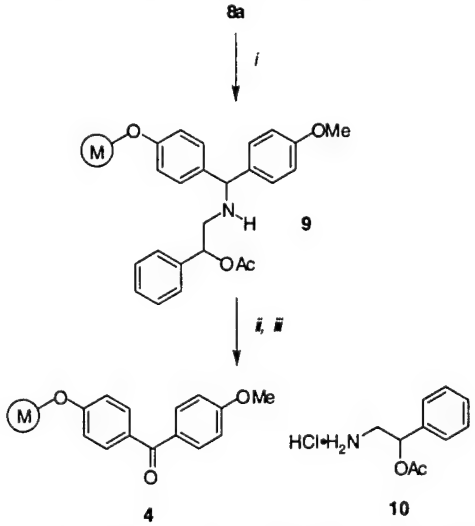
Preparation of 4,4'-dialkoxy-diphenylketimine on a solid support was achieved using both Merrifield resin **1** and Wang resin **19** (**Scheme 1**).[4] Displacement of chloride with the cesium salt of 4-cyanophenol gave **2** (98% based on elemental analysis for Cl^-). Grignard addition to **2** and subsequent quenching with anhydrous methanol afforded the ketimine resin **3**. The reactions were monitored by FT-IR (KBr Pellet; nitrile at 2221 cm^{-1}). Hydrolysis of **3** to give **4** under acidic conditions and can be detected by the presence of a carbonyl stretch at 1645 cm^{-1}. Ketimine resin **20** was synthesized in the same manner from the bromo-Wang resin **19**.

Several substituted and unsubstituted aziridines were synthesized and opened to yield β-amino esters.[5] A toluene suspension of resin **3** and the tosylate-salts of 1-amino-2-propanol or 1-phenyl-ethanolamine were condensed to yield resin-bound Schiff bases **5a** and **5c**. (**Scheme 2**) FT-IR was not adequate for analysis of this reaction. Instead, ^{13}C-NMR spectra were used to characterize the resins, and were compared to solution phase models, which were prepared in parallel with the resin-supported materials. Treatment with $LiBH_4$ and MeOH in THF reduced **5a** and **5c** to benzhydrylamines **7a** and **7c**.[6] This procedure was also used to reduce the resin bound glycine ethyl ester Schiff base **6** to the unsubstituted β-amino alcohol **7b**. Dehydration of the aminoalcohols to give aziridines **8a**, **8b** and **8c** was accomplished by treatment with $Ph_3P{\bullet}Br_2$.[7]



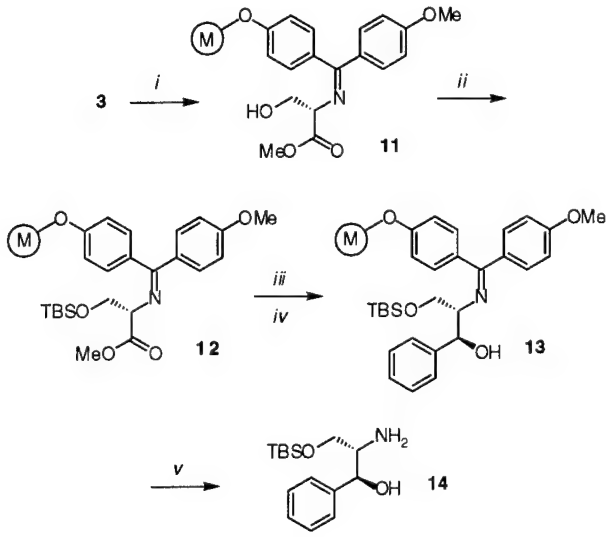
Scheme 2. Reagents: *i.* H_2NCH_2CRHOH / TsOH / $PhCH_3$ / 60°C, *ii.* $EtO_2CCH_2NH_2{\bullet}HCl$ / CH_2Cl_2 / RT, *iii.* $LiBH_4$ / MeOH / THF / reflux, *iv.* $Ph_3P{\bullet}Br_2$ / NEt_3 / CH_2Cl_2 / 0°C —> RT

SN^1-Like opening of aziridine **8a** was accomplished under acidic conditions[8] (**Scheme 3**) to yield the benzhydryl-linked secondary acetate **9**. Oxidation back to the Schiff base with DDQ,[9] followed by hydrolysis with aqueous HCl in methanol yielded **10**[10] and the resin-bound ketone **4**. Interestingly, under the same conditions **8b** opened in an SN^2-like fashion to provide a 6:1 mixture of β-amino acetates, favoring the primary acetate.

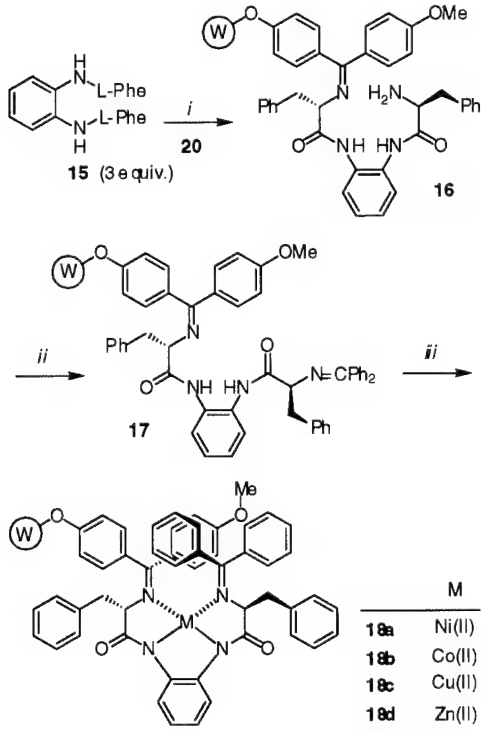


Scheme 3. Reagents: *i.* HOAc / CH_3CN, *ii.* DDQ / CH_2Cl_2, *iii.* H_3O^+ / MeOH

Transimination[11] of **3** with methyl-L-serinate hydrochloride yielded the resin-bound intermediate **11**. (**Scheme 4**) Protection as the silyl ether was achieved with TBS-Cl, and the protected resin-bound serine **12** was treated with an equimolar mixture of iBu_2AlH and iBu_3Al (*e.g.* iBu_5Al_2H) at –78°C. Addition of PhMgBr, followed by warming to RT yielded the amino alcohol **13**.[12] Cleavage with PPTS in aqueous THF provided **14**, which was predominately *threo* (10:1) by integration of the crude ^1H NMR spectrum and by capillary GC.



Scheme 4. Reagents: *i.* L-Serine Methyl Ester•HCl / CH_2Cl_2, *ii.* TBDMS-Cl / imidazole / DMF / RT, *iii.* iBu_5Al_2H / CH_2Cl_2 / -78°C, *iv.* PhMgBr / -78°—> 0°C, *v.* C_5H_5N•$HOSO_2PhCH_3$ / H_2O / THF.



Scheme 5. Reagents: *i.* TsOH / $PhCH_3$ / 80°C / 24 h, *ii.* Ph_2C=NH / TsOH / $PhCH_3$ / 80°C / 24 h, *iii.* a) $NiBr_2$ / NEt_3 / THF / reflux, b) $CoCl_2$ / DBU / DMF / 50°C, c) $CuCl_2$ / DBU / DMF / 50°C, d) Et_2Zn / THF / reflux.

Hugo Schiff reported the first examples of imine-metal complexes in 1869.[13] Based on X-ray work with Ni(II) complexes,[14] which showed shortened N-Ni bond lengths for *para*-methoxy-substituted imines,[15] it was hoped that the presence of the para-alkoxy substituent used to attach the ketimine to the resin would enhance the metal-binding ability of the imines. The Wang resin **20** was used as a support (**Scheme 5**) to attach the bifurcated dipeptide **15**. Attachment was followed by FT-IR (amide C=O stretch at 1681 cm^{-1}). Capping of **16** with diphenylketimine gave the supported ligand **17**.

The ligand-bearing resin **17** has been shown to bind the metals Ni^{II}, Co^{II}, Cu^{II}, and Zn^{II} efficiently. (**Figure 1**) Complexation of Ni^{II} caused the pale yellow resin to change color and sink to the bottom of the reaction vessel, indicating a profound change in density. Upon complexation, Co^{II} and Cu^{II} showed similar behavior, but a different color change. The oxygen- and moisture-sensitive Zn^{II} complex was colorless, and was prepared from Et_2Zn under argon.

Enantioselective 1,2-additions to carbonyls have been achieved with the Wang-supported catalyst **18d**.[16] The Et_2Zn-activated resin could be used multiple times without reduction in yield or the enantioselectivity.[17] The yields and e.e.'s were only slightly diminished relative to the solution-phase catalyst for each case studied. In summary, the benzophenone imine linkage can be used for a variety of purposes. Its utility for the synthesis of small organic molecules, the reductive alkylation of resin-bound imino esters, and for catalysis have been demonstrated. Its use in natural product synthesis is currently under investigation.

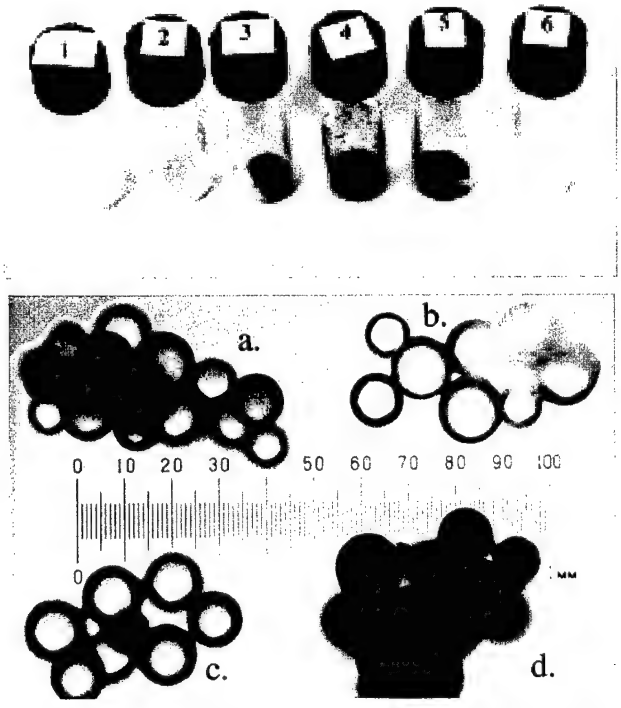


Figure 1. Above— Photograph of resins: 1) Wang diphenylketimine **20**, 2) Wang L-Phe-L-Phe ligand **17**, 3) Ni(II)-loaded resin **18a**, 4) Cu(II)-loaded resin **18c**, 5) Co(II)-loaded resin **18b**, 6) Resin **7b**. Below— Composite photomicrograph of resins: a) Wang diphenylketimine **20**, b) Wang L-Phe-L-Phe ligand **17**, c) Ni(II)-loaded resin **18a**, d) Cu(II)-loaded resin **18c**.

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Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/angewandte/ or from the author.

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Glycosyltransferase Inhibitors: Synthesis of D-*threo*-PDMP, L-*threo*-PDMP, and Other Brain Glucosylceramide Synthase Inhibitors from D- or L-Serine

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The synthesis of enantiomerically pure (1*S*,2*S*)-1-phenyl-2-decanoylamino-3-*N*-morpholino-1-propanol (L-*threo*-PDMP) (**1a**) from L-serine, and the enantiomer (1*R*,2*R*)-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-*threo*-PDMP] (**1b**) from D-serine is reported. Reductive alkylation of the fully protected O'Donnell's Schiff base (**3b**) derived from D-serine provided the β-amino alcohol **5b** in high yield and excellent selectivity, which yielded optically pure **1b** in high yield after six steps. Three other D-*threo*-PDMP analogues with various amine groups have been synthesized using the same methodology, including the more potent pyrrolidine compound D-*threo*-PDPP (**1e**). A key feature of the synthesis is the isolation of tosylate (**8b**), which allows for the divergent synthesis of many analogues from a common advanced intermediate. The synthesis is amenable to large-scale production of D-*threo*-PDMP, L-*threo*-PDMP, and similar compounds.

The regulation of glycosphingolipids (GSLs) is critical for normal cellular function and is an important means of regulating cell−cell communication, cell adhesion and proliferation, neuronal growth, cell transformation, tumor progression, and immune response.[1] Biochemical tools (potent and selective enzyme inhibitors) are required for the exploration of GSL function as well as for new therapeutic approaches. Since its discovery by Vunnam and Radin in 1980,[2] the drug D-*threo*-PDMP (**1b**) (D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol) has been shown to be a potent inhibitor of the glucosyltransferase responsible for attachment of the first sugar to ceramide, glucosylceramide synthase.[3] The syntheses reported by Inokuchi and Radin[4] in 1987, Ganem and colleagues[5] in 1994, and Ogawa et al.[6] in 1997 all have significant limitations. This compound and its congeners have broad clinical application not only as glycosphingolipid modulation agents,[7] but also as antitumor agents.[8]

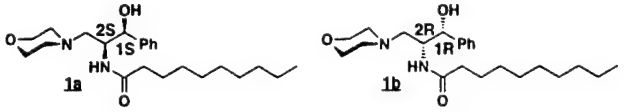

Figure 1.

The administration of D-*threo*-PDMP, **1b**, has interesting developmental consequences caused by inhibition of the biosynthesis of gangliosides.[9] More recently, D-*threo*-PPMP, the hexadecanoyl analogue of **1b**, has been demonstrated to reverse multidrug resistance in cancer cells.[10] The best-studied analogue, D-*threo*-PDMP (**1b**), inhibits glucosylceramide synthase, resulting in the *decreased* de novo synthesis of glucosylceramide and down-regulation of ganglioside biosynthesis.[11] Interestingly enough, the enantiomer L-*threo*-PDMP (**1a**) *increased* the biosynthesis of gangliosides, leading to enhanced synapse formation and increased memory retention in rats.[12] Although it seems likely that one or more sphingosine-, sphingomyelin-, ceramide-, or glycosphingolipid-processing enzymes is affected by the L-compounds, the precise molecular target or targets of **1a** remain murky. These facts dictate that an enantio-

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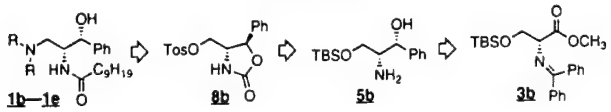
Scheme 1



merically pure synthesis of **1a**, **1b**, and related analogues is required. Ideally, a single advanced intermediate should provide for the synthesis of numerous structural analogues without reworking the entire synthetic approach for each analogue.

Our strategy focuses on the stereoselective reductive alkylation of a serine-derived Schiff base ester such as **3b** (Scheme 1). This provides the (1*R*,2*R*) diastereomer in high yield without racemization, and with good threo-diastereoselectivity.[13,14] Easy separation of the desired threo isomers on SiO_2 has been shown to be general.[15,16] Our hope was that the PDMP molecule could be generated from the carbamate tosylate, **8b**,[17] which could be obtained from the corresponding β-amino diol, **5b**. Thus, diverse cyclic secondary amines and fatty acids could be introduced at either end to produce the various PDMP analogues from a single advanced intermediate.

Initially, the Schiff base derived from L-serine was taken through to the less-studied isomer L-*threo*-PDMP **1a** without isolation of the tosylate intermediate **8a**. Subsequently, the D-form of *threo*-PDMP (**1b**), as well as a series of D-threo analogues **1c**, **1d**, and **1e**, were synthesized from the enantiomeric intermediate **8b**, which was easily purified by crystallization.

Imino ester **3a**, obtained in three steps from L-serine,[13,14b] was sequentially treated with *i*-Bu_5Al_2H (1:1 mixture of *i*-Bu_2AlH and *i*-Bu_3Al in hexanes) followed by PhMgBr (3.0 M in Et_2O) in CH_2Cl_2 at −78 °C to yield the threo imino alcohol **4a** as an oil in 69% yield and 8:1 selectivity. Because of imine−oxazoline tautomerization,[16] the minor erythro product was easily removed by flash chromatography[17] along with small amounts of over-reduced primary alcohol (Scheme 2).

Treatment of **4a** with pyridinium *p*-toluenesulfonate in THF:H_2O (10:1) cleanly removed the Schiff base to afford threo β-amino alcohol **5a** in 72% yield. The carbamate moiety was necessary to protect the amine, since attempts to displace the primary tosylates and mesylates of several threo-ceramides led to intramolecular attack of the amide on the tosylate, resulting in formation of cyclic imino ethers. Thus, the carbamate "ties back" the amide carbonyl, preventing intramolecular displacement of the tosylate (oxazoline formation[15]), while protecting the benzylic alcohol at the same time.[18] Treatment with either carbonyl diimidazole or triphosgene [$Cl_3CO(CO)OCCl_3$] afforded the silyl carbamate **6a** in 70% yield as a waxy solid. Removal of the silyl group was achieved with 10% aqueous HF in acetonitrile (10:1 CH_3CN/49% HF in H_2O) to yield crystalline carbamate alcohol **7a** in 92% yield. Although chlorides have been generated under similar conditions,[19] tosylation of the primary alcohol using tosyl chloride in pyridine and DMAP cleanly furnished **8a** in 97% yield as a white crystalline solid (mp 116−117 °C).

For L-*threo*-PDMP, **1a**, tosylate formation and subsequent displacement with 10 equivalents of morpholine in THF at reflux for 28 h was done in the same pot to yield the morpholino carbamate **9a** as an oil in 90% yield. Thus, isolation of the tosylate **8a** is not necessary. Removal of the carbamate was achieved by treatment with 1 M KOH in a 4:1 mixture of MeOH:H_2O at 65 °C for 36 h, affording the morpholino amino alcohol **10a** in 80% yield as a colorless oil. The fatty amide chain was then introduced using either *p*-nitrophenyl decanoate or pentafluorophenyl decanoate as the acylating agent and 10 mole-% HOBt in dry pyridine. After extraction with 1 N NaOH to remove *p*-nitrophenol or pentafluorophenol, and flash chromatography, the desired drug enantiomer **1a** was obtained as an oil in 90% yield (Scheme 3).

Starting with D-serine, the synthesis was repeated to produce enantiomeric Schiff base **3b**, which in turn provided the enantiomeric tosylate **8b** in crystalline form. Displacement of the tosylate with cyclic amines provided the carbamate amines **9b**−**9e** (Scheme 4). Similarly, each of the other alkylated amines was deprotected and acylated as before to provide the isomeric D-*threo*-PDMP **1b**, as well as the depicted structural analogues. Analogue **1e** is of particular note because reported studies (with enantiomeric mixtures) suggest that this compound has four times the activity of **1b** against glycosylceramide synthase.[9b]

Now that an efficient enantioselective synthesis has been established, diverse analogues of D-*threo*-PDMP can be easily prepared to explore the nature of the interaction with glucosylceramide synthase and other sphingosine- and ceramide-processing enzymes. Because a number of the intermediates are crystalline and the protecting group manipulations are straightforward, it is expected that this route could also be optimized for large-scale synthesis of these compounds. Our ongoing work will involve the introduction of more complex secondary amines at the glycosphingosine-like headgroup, changes in the fatty acid amide chain of the ceramide, and variation of the carbanion source during reductive alkylation to produce alterations in the sphingosine moiety.

Experimental Section

General Information. All air- and moisture-sensitive reactions were performed under an argon atmosphere in flame-dried reaction flasks. THF was dried and deoxygenated over $Ph_2C{=}O/K°$. CH_2Cl_2 (dichloromethane) was dried over P_2O_5, CH_3CN (acetonitrile) was dried over CaH_2, and all solvents were freshly distilled under an argon atmosphere before use. For flash chromatography, 400−230 mesh silica gel 60 (E. Merck no. 9385) was used. All compounds described were >95% pure by ^1H and ^{13}C NMR, and purity was confirmed by high-resolution fast atom bombardment (FAB) mass spectrometry in most cases, and by CHN analysis for one of the final products. The ^1H and ^{13}C NMR spectra were obtained on either a Bruker WM 250 MHz or a Bruker 500 MHz spectrometer. For ^{13}C, spectra were taken at 62.9 MHz in the form

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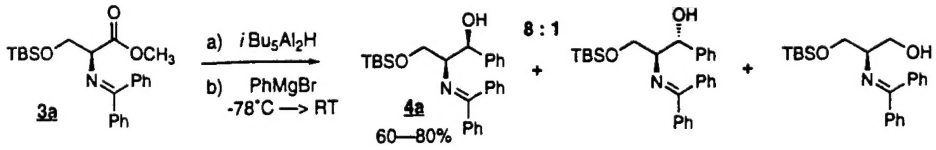
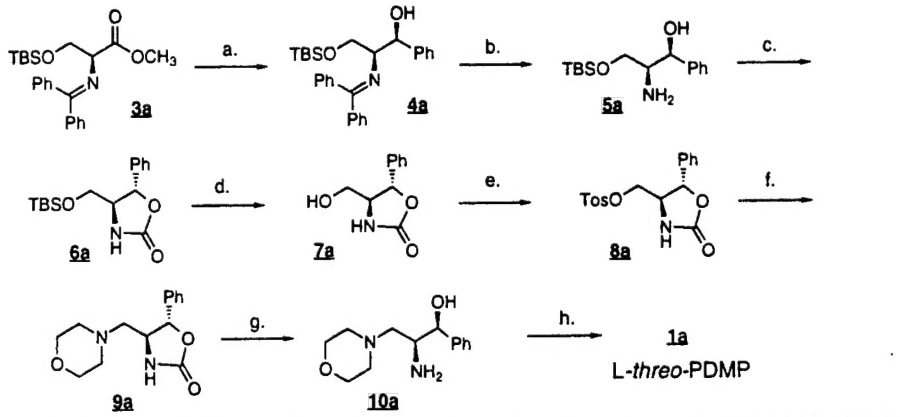
Scheme 2

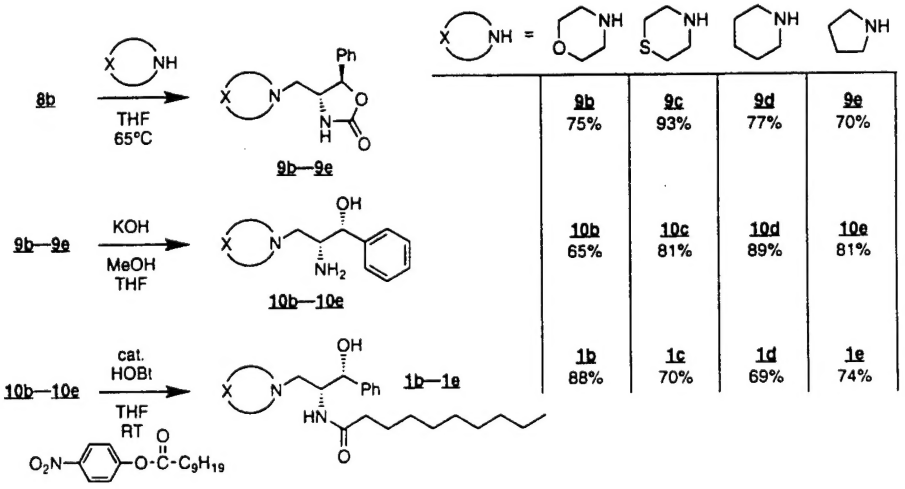


Scheme 3



a) i. $iBu_2AlH{\cdot}iBu_3Al$ / hexanes / -78°C ii. C_6H_5MgBr b) pyridine•HOTs / THF / H_2O c) $Cl_3COCOOCCl_3$ / THF d) HF / H_2O / CH_3CN e) TosCl / pyridine f) Morpholine / THF / Δ g) KOH (aq.)/Δ h) NO_2-C_6H_4-O_2C-C_9H_{19}

Scheme 4



X-NH =	O-NH	S-NH	NH	NH
	9b 75%	9c 93%	9d 77%	9e 70%
	10b 65%	10c 81%	10d 89%	10e 81%
	1b 88%	1c 70%	1d 69%	1e 74%

of APTs (attached proton test spectra). Chemical shifts are reported in δ vs Me_4Si in ^1H spectra and vs $CDCl_3$ in ^{13}C spectra. Infrared spectra were taken on a Nicolet Impact-400D FT-IR. Optical rotations were taken on a Jasco DIP-1000 polarimeter using the Na^D-line. All melting points were taken on a Hoover capillary melting point apparatus and are uncorreced. Elemental analyses were performed by Desert Analytics of Tucson, Arizona.

Methyl-*O*-(*tert*-butyldimethylsilyl)-*N*-(diphenylmethylene)-D-serinate (3b). Methyl-*N*-(diphenylmethylene)-D-serinate (28.00 g, 0.099 mol) was dried over P_2O_5 in vacuo and added to a RB-flask and dry dimethyl formamide (DMF) (90 mL) was added. TBDMS–Cl (23.83 g, 0.16 mol) and imidazole (17.5 g, 0.257 mol) were added in one portion and stirred at room temperature (RT) under argon for 24 h. The sample was then poured over ether (200 mL) and washed 2× with 1% $NaHCO_3$. The organic layer was pooled, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resultant oil solidified on standing to give 33.60 g of a crystalline mass in 86% yield. MP: 56–58 °C. $[\alpha]^{25}_D = +100.68°$ (c = 0.76, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 7.66–7.20 (Ar–*H*, m, 10H), 4.32 (αC*H*, dd, J = 7.58, 5.4 Hz, 1H), 4.13 (β*H*, dd, J = 9.75, 5.4 Hz, 1H), 3.94 (β′*H*, dd, J = 9.73, 7.63 Hz, 1H), 3.70 (OCH_3, s, 3H), 0.84 [(CH_3)$_3$C–, s, 9H], 0.02 (CH_3–Si–, s, 3H), −0.0044 (CH_3′–Si–, s, 3H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 171.5, 171.0, 139.5, 136.1, 130.2, 128.8, 128.6, 128.3, 128.27, 127.9, 67.7, 51.9, 25.7, 18.2, −5.4, −5.5. IR (CH_2Cl_2): 3060, 2952, 1740, 1625 cm^{-1}.

(1*R*,2*R*)-(−)-2-Amino-*N*-(diphenylmethylene)-3-*O*-(*tert*-butyldimethylsilyl)-1-phenylpropane-1,3-diol (4b). Fully protected D-serine **3b** (20.0 g, 0.05 mol) was added to CH_2Cl_2 (500 mL) in a long-necked addition flask under argon and cooled to −78 °C. To this solution was added i-$Bu_2AlH{\cdot}i$-Bu_3Al (0.05 mol of each in 100.60 mL of hexanes) at −78 °C over 90 min. The solution turned yellow upon addition and was allowed to stir for 60 min. Next, PhMgBr in ether (50.3 mL, 3 equiv, 0.15 mol) was added dropwise at −78 °C over 130 min and the resulting solution was allowed to stir overnight and slowly warm from −78 to 0 °C, turning a deep orange-yellow color. After completion of reaction the flask was cooled to 0 °C and concentrated $NaHCO_3$ (~10 mL) was added dropwise slowly to quench the reaction. The reaction turned a light milky yellow upon quenching, and the solution was diluted with CH_2Cl_2 (400 mL), the phases separated, and the crude was washed 3× with concentrated $NaHCO_3$, followed by brine and dried over $MgSO_4$. Silica gel flash chromatography (5% EtOAc:hexanes, R_f = 0.63) yielded 15.60 g of pure threo product as a colorless oil in 70% yield. $[\alpha]^{25}_D = -109°$

(c = 1.70, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 7.83−7.14 (Ar−H, m, 15H), 4.94 (CH−O, d, J = 6.23 Hz, 1H), 3.86 (βH, dd, J = 10.73, 3.05 Hz, 1H), 3.64 ($\beta' H$, dd, J = 12.08, 1.43 Hz, 1H), 3.14 (αCH, dt, J = 8.25 Hz, 1H), 0.85 [(CH_3)$_3$C−, s, 9H], 0.08 (CH_3−Si−, s, 3H), 0.04 (CH_3'−Si−, s, 3H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 145.6, 145.3, 140.4, 128.44, 128.33, 128.12, 128.07, 127.8, 127.6, 127.5, 127.3, 126.9, 126.6, 125.9, 125.7, 100.1, 81.9, 68.3, 59.1, 25.7, 18.1, −5.5. IR (CH_2Cl_2): 3061, 2953, 1450 cm^{-1}.

(1*R*,2*R*)-(+)-2-amino-3-*O*-(*tert*-butyldimethylsilyl)-1-phenylpropane-1,3-diol (5b). Threo product **4b** (12.90 g, 2.89 mmol) was dissolved in THF (200 mL) and H_2O (20 mL). Pyridinium p-toluenesulfonate (14.55 g, 5.79 mmol, 2 equiv) was added and reacted for 4.5 h, the solvent was removed in vacuo, and the residue was redissolved in CH_2Cl_2 (150 mL) and washed with concentrated $NaHCO_3$ (3 × 50 mL), brine, and dried over K_2CO_3. The crude product was then chromatographed on silica gel (9:1, CH_2Cl_2:MeOH, R_f = 0.5) yielding 6.54 g as a colorless oil in 80% yield. $[\alpha]^{25}_D$ = +3.14° (c = 1.34, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 7.36−7.27 (Ar−H, m, 5H), 4.64 (CH−OH, d, J = 5.26 Hz, 1H), 3.65 (βH, dd, J = 10.07, 4.12 Hz, 1H), 3.58 ($\beta' H$, dd, J = 10.11, 5.00 Hz, 1H), 2.21 (OH, bs, 1H), 0.91 [(CH_3)$_3$C−, s, 9H] 0.06 [(CH_3)$_2$−Si−, s, 6H]. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 142.4, 128.2, 127.3, 126.2, 73.9, 65.0, 58.3, 25.8, 18.1, −5.60. IR (neat): 3444, 2956, 912, 740 cm^{-1}.

(4*R*,5*R*)-(+)-4-(*tert*-Butyldimethylsilyloxymethyl)-5-phenyloxazolidin-2-one (6b). Amino alcohol **5b** (1.92 g, 6.82 mmol) was dissolved in 20 mL of dry CH_2Cl_2 under argon and cooled to 0 °C. Triethylamine was then added in one portion (2.87 mL, 20.6 mmol) at 0 °C. A solution of triphosgene (682.5 mg, 2.3 mmol, 0.33 equiv) in CH_2Cl_2 (20 mL) was added dropwise over 60 min. After 4 h the reaction was complete, and all solvent was removed in vacuo. The residue was then redissolved in Et_2O, filtered, and washed with concentrated $NaHCO_3$ (3 × 100 mL) and dried over $MgSO_4$. Flash chromatography (3:2 hexanes:EtOAc, R_f = 0.57) yielded 1.52 g as a waxy solid in 72.8% yield. MP: 88−90 °C. $[\alpha]^{25}_D$ = +31.1° (c = 0.405, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 7.42−7.34 (Ar−H, m, 5H), 6.58 (NH, bs, 1H), 5.35 (CH−OH, d, J = 4.63 Hz, 1H), 3.79 (CH−NH, p, J = 5.78, 4.98 Hz, 1H), 3.74 (βCH_2, d, J = 4.75 Hz, 2H), 0.91 [(CH_3)$_3$C−Si, s, 9H], 0.094 (CH_3−Si, s, 6H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 159.7, 139.0, 128.6, 128.4, 125.3, 79.8, 64.0, 61.5, 25.6, 18.0, −5.6. IR (CH_2Cl_2): 3416, 2955, 1757 cm^{-1}. HRMS $C_{16}H_{26}NO_3Si$ [M + H] calcd. 308.1681, found 308.1682.

(4*R*,5*R*)-(+)-4-(Hydroxymethyl)-5-phenyloxazolidin-2-one (7b). Carbamate **6b** (1.00 g, 3.25 mmol) was dissolved in 20 mL of acetonitrile and HF (49% aqueous, 0.5 mL, 12.25 mmol, 3.8 equiv) was added via syringe at RT and stirred for 75 min. After TLC showed full conversion, solvent was removed in vacuo. The crude product was redissolved in 9:1 CH_2Cl_2:MeOH and passed through a plug of silica gel (R_f = 0.44). Isolation and concentration yielded 0.60 g as white crystals in 95% yield. MP: 96−97 °C. $[\alpha]^{25}_D$ = +44.0° (c = 0.345, MeOH). ^1H NMR (250 MHz, CD_3OD + D_2O): δ 7.37 (Ar−H, m, 5H), 5.37 (CH−OD, d, J = 5.6 Hz, 1H), 3.83 (βCH_2, dt, J = 9.41, 3.21 Hz, 2H), 3.67 (CH−ND_2, dd, J = 12.61, 5.46 Hz, 1H). ^{13}C NMR (62.5 MHz, CD_3OD): δ 159.9, 138.3, 128.9, 125.7, 79.8, 62.7, 61.8. IR (CH_2Cl_2): 3292, 3057, 1707, 1268 cm^{-1}. MS (FAB) [M + H] for $C_{10}H_{12}NO_3$; HRMS calcd. 194.0817, found 194.0814.

(4*R*,5*R*)-(+)-4-(4′-*p*-Toluenesulfonyloxymethyl)-5-phenyloxazolidin-2-one (8b). Alcohol **7b** (330.8 mg, 1.71 mmol) was dissolved in dry pyridine (2.0 mL) and tosyl chloride (502.5 mg, 2.635 mmol, 1.5 equiv) was added in one portion. The resulting mixture was reacted at RT for 4 h, the solvent was removed in vacuo and the crude was purified by flash chromatography (EtOAc:hexanes 1:1, R_f = 0.33), yielding 585.5 mg as a white crystalline solid in 98.5% yield. MP: 116−117 °C. $[\alpha]^{25}_D$ = +39.6° (c = 0.75, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 7.80 (Ar−H, 2H, d, J = 8.3 Hz), 7.41−7.27 (Ar−H, m, 7H), 6.28 (NH, bs, 1H), 5.23 (CH−OPh, d, J = 5.45 Hz, 1H), 4.15 (CH_2−OTos, dd, J = 5.22, 5.41 Hz, 2H), 3.96 (CH−NH, dd, J = 5.32, 5.24 Hz, 1H), 2.46 (CH_3, s, 3H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 158.5, 145.6, 137.4, 131.9, 130.1, 129.2, 129.0, 127.9, 125.5, 79.3, 68.9, 58.8, 21.6. IR (CH_2Cl_2): 3018, 1771, 1221, 758 cm^{-1}. MS (FAB) [M + H] for $C_{17}H_{18}O_5NS$; HRMS calcd. 348.0906, found 348.0913.

(4*R*,5*R*)-(+)-4-[(*N*-Morpholino)methyl]-5-phenyloxazolidin-2-one (9b). Tosylate **8b** (585.5 mg, 1.69 mmol) was dissolved in THF (5.0 mL) and morpholine (0.44 mL, 5.0 mmol, 3 equiv) was added, and the resultant mixture was heated to reflux for 34 h. THF was removed in vacuo, the residue was dissolved in CH_2Cl_2 and extracted with $NaHCO_3$, and flash chromatography (CH_2Cl_2:MeOH 20:1, R_f = 0.34) yielded 335 mg as a colorless oil in 75.2% yield. $[\alpha]^{25}_D$ = +36.5° (c = 0.325, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 7.38 (Ar−H, m, 5H), 6.24 (NH, bs, 1H), 5.22 (CH−OPh, d, J = 5.65 Hz, 1H), 3.84 (CH−NH, dd, J = 6.74, 6.26 Hz, 1H), 3.67 (CH_2−O_{MOR}, t, 4.59 Hz, 4H), 2.74 (βCH_2−N, ddd, 4.74, 3.46 Hz, 2H), 2.46 (CH_2−N_{MOR}, tq, 13.2, 4,6 Hz, 4H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 159.0, 138.5, 128.5, 125.4, 81.4, 66.4, 62.1, 57.4, 53.6. IR (neat): 3434, 3018, 1759 cm^{-1}. HRMS $C_{14}H_{19}N_2O_3$ [M + H] calcd. 263.1396, found 263.1396.

(4*R*,5*R*)-(+)-4-[(*N*-Thiomorpholino)methyl]-5-phenyloxazolidin-2-one (9c). Tosylate **8b** (235.5 mg, 0.68 mmol) was dissolved in THF (5.0 mL) and thiomorpholine (0.52 mL, 5.2 mmol, 7.7 equiv) was added, and the resultant mixture was heated to reflux for 47 h. THF was removed in vacuo, the residue was dissolved in CH_2Cl_2 and extracted with $NaHCO_3$, and flash chromatography (CH_2Cl_2:MeOH 20:1, R_f = 0.55) yielded 175 mg as a colorless oil in 93.1% yield. $[\alpha]^{25}_D$ = +30.9° (c = 0.25, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 7.42−7.35 (Ar−H, m, 5H), 5.60 (NH, bs, 1H), 5.17 (CH−OPh, d, J = 5.53 Hz, 1H), 3.82 (CH−NH, q, J = 7.65 Hz, 1H), 2.81−2.77 (m, 2H), 2.72−2.68 (m, 2H), 2.66−2.56 (m, 6H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 159.1, 138.6, 128.7, 125.5, 81.5, 62.6, 57.7, 55.3, 27.7. IR (neat): 3426, 2923, 1754, 1657 cm^{-1}. HRMS $C_{14}H_{19}N_2O_2S$ [M + H] calcd. 279.1167, found 279.1179.

(4*R*,5*R*)-(+)-4-[(*N*-Piperidino)methyl]-5-phenyloxazolidin-2-one (9d). Tosylate **8b** (330 mg, 0.95 mmol) was dissolved in THF (5.0 mL) and piperidine (0.94 mL, 9.5 mmol, 10 equiv) was added, and the resultant mixture was heated to reflux for 16 h. THF was removed in vacuo, the residue was dissolved in CH_2Cl_2 and extracted with $NaHCO_3$, and flash chromatography (CH_2Cl_2:MeOH 20:1, R_f = 0.35) yielded 196 mg as a colorless oil in 76.7% yield. $[\alpha]^{25}_D$ = +40.7° (c = 0.245, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 7.38 (Ar−H, m, 5H), 6.24 (NH, bs, 1H), 5.22 (CH−OPh, d, J = 5.65 Hz, 1H), 3.84 (CH−NH, dd, J = 6.74, 6.26 Hz, 1H), 3.67 (CH_2−O_{MOR}, t, 4.59 Hz, 4H), 2.74 (βCH_2−N, ddd, 4.74, 3.46 Hz, 2H), 2.46 (CH_2−N_{MOR}, tq, 13.2, 4.6 Hz, 4H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 159.0, 138.5, 128.5, 125.4, 81.4, 66.4, 62.1, 57.4, 53.6. IR (neat): 3419, 3066, 2935, 1755, 1655 cm^{-1}. HRMS $C_{15}H_{21}N_2O_2$ [M + H] calcd. 261.1603, found 261.1606.

(4*R*,5*R*)-(+)-4-[(*N*-Pyrrolidino)methyl]-5-phenyloxazolidin-2-one (9e). Tosylate **8b** (187.6 g, 0.54 mmol) was dissolved in THF (5.0 mL) and pyrrolidine (0.45 mL, 5.4 mmol, 10 equiv) was added and heated to reflux for 11 h. THF was removed in vacuo, the residue was dissolved in CH_2Cl_2 and extracted with $NaHCO_3$, and flash chromatography (CH_2Cl_2:MeOH 20:1, R_f = 0.35) yielded 87.9 mg as a colorless oil in 70.1% yield. $[\alpha]^{25}_D$ = +43.8° (c = 0.665, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 7.42−7.35 (Ar−H, m, 5H), 5.59 (NH, bs, 1H), 5.19 (CH−OPh, d, J = 5.73 Hz, 1H), 3.80 (CH−NH, dt, J = 6.74, 8.55 Hz, 1H), 2.84 (βH, dd, J = 12.02, 8.95 Hz, 1H), 2.65 ($\beta' H$, dd, J = 12.13, 4.97 Hz, 1H), 2.59 (αCH_{2PYR}, m, 2H), 2.51 ($\alpha' CH_{2PYR}$, m, 2H), 1.78 (βCH_{2PYR}, m, 4H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 158.9, 138.7, 128.7, 128.6, 125.6, 81.4, 59.9, 59.3, 54.2, 23.4. IR (neat): 3390, 2960, 1755 cm^{-1}. HRMS $C_{14}H_{19}N_2O_2$ [M + H] calcd. 247.1447, found 247.1447.

(1*R*,2*R*)-(+)-2-Amino-3-(*N*-morpholino)-1-phenyl-1-ol (10b). Carbamate **9b** (330 g, 1.26 mmol) was dissolved in MeOH:H_2O (4:1) and treated with) 2 M KOH (5.0 mL) and heated under reflux conditions at 70 °C, and the reaction progress was monitored by TLC. After 16 h the sample was concentrated in vacuo and redissolved in CH_2Cl_2 and washed with concentrated $NaHCO_3$. Passing the sample through silica gel using 20% MeOH:CH_2Cl_2 yielded 192.4 mg as a colorless

oil in 65.4% yield. $[\alpha]^{25}_D = +5.86°$ ($c = 0.25$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 7.38–7.25 (Ar–*H*, m, 5H), 4.55 (C*H*–OH, d, $J = 3.29$ Hz, 1H), 3.70 (CH_2–O_{MOR}, t, $J = 4.6$ Hz, 4H), 3.21 (C*H*–NH_2, ddd, $J = 6.57$, 5.33 Hz, 1H), 2.56 (βH, dd, $J = 11.28$, 4.23 Hz, 1H), 2.36 ($\beta' H$, dd, $J = 12.58$, 5.44 Hz, 1H), 2.45 (CH_2–N_{MOR}, m, 4H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 142.5, 128.2, 127.2, 125.9, 75.1, 66.9, 61.9, 53.9, 52.7. IR (neat): 3450, 3018, 1641 cm^{-1}. HRMS $C_{13}H_{21}N_2O_2$ [M + H] calcd. 237.1603, found 237.1603.

(1*R*,2*R*)-(+)-2-Amino-1-phenyl-3-(*N*-thiomorpholino)-1-ol (10c). Carbamate **9c** (105.4 mg, 0.399 mmol) was dissolved in MeOH:H_2O (4:1) and treated with) 2 M KOH (5.0 mL) and heated under reflux conditions at 70 °C, and the reaction progress was monitored by TLC. After 18 h the sample was concentrated in vacuo and redissolved in CH_2Cl_2 and washed with concentrated $NaHCO_3$. Passing the sample through silica gel using 20% MeOH:CH_2Cl_2 yielded 100.6 mg as a colorless oil in 81.3% yield. $[\alpha]^{25}_D = +5.23°$ ($c = 0.32$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$ + D_2O): δ 7.37–7.26 (Ar–*H*, m, 5H), 4.54 (C*H*–OH, d, $J = 3.17$ Hz 1H), 3.22 (C*H*–NH_2, dt, $J = 3.4$, 6.87 Hz 1H), 2.82 (CH_2–N_{TM}, m, 2H), 2.71 (CH_2'–N_{TM}, m, 2H), 2.67 (CH_2–S_{TM}, m, 4H), 2.44 (βCH_2, d, $J = 6.66$ Hz). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 142.5, 128.2, 127.2, 125.9, 75.1, 62.2, 55.4, 52.9, 27.9.

(1*R*,2*R*)-(+)-2-Amino-1-phenyl-3-(*N*-piperidino)-1-ol (10d). Carbamate **9d** (167 mg, 0.64 mmol) was dissolved in MeOH:H_2O (4:1) and treated with) 2 M KOH (5.0 mL) and heated under reflux conditions at 70 °C, and the reaction progress was monitored by TLC. After 16 h the sample was concentrated in vacuo and redissolved in CH_2Cl_2 and washed with concentrated $NaHCO_3$. Passing the sample through silica gel using 20% MeOH:CH_2Cl_2 yielded 150 mg as a colorless oil in 99.7% yield. $[\alpha]^{25}_D = -6.35°$ ($c = 0.29$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$ + D_2O): δ 7.29–7.14 (Ar–*H*, m, 5H), 4.55 (C*H*–OH, d, $J = 3.37$ Hz 1H), 3.16 (C*H*–NH_2, dt, 6.72, 3.36 Hz 1H), 2.36 (αCH_{2PIP}, m, 4H), 2.32 (βH, dd, $J = 12.8$, 6.95 Hz, 1H), 2.22 ($\beta' H$, dd, $J = 12.7$, 6.63 Hz, 1H), 1.50 (βCH_{2PIP}, p, $J = 5.6$ Hz, 4H), 1.36 (γCH_{2PIP}, bp, $J = 4.975$ Hz, 2H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 142.3, 127.9, 126.9, 126.2, 76.0, 62.4, 55.1, 52.3, 25.9, 24.1. IR (neat): 3373, 3017, 2933, 1452 cm^{-1}. HRMS $C_{14}H_{23}N_2O$ [M + H] calcd. 235.1810, found 235.1812.

(1*R*,2*R*)-(+)-2-Amino-1-phenyl-3-(*N*-pyrrolidino)-1-ol (10e). Carbamate **9e** (88 mg, 0.036 mmol) was dissolved in MeOH:H_2O (4:1) and treated with) 2 M KOH (5.0 mL) and heated to 70 °C under reflux conditions and the reaction progress was monitored by TLC. After 16 h the sample was concentrated in vacuo and redissolved in CH_2Cl_2 and washed with concentrated $NaHCO_3$. Passing the sample through silica gel using 20% MeOH:CH_2Cl_2 yielded 79 mg as a colorless oil in 80.7% yield. $[\alpha]^{25}_D = +3.51°$ ($c = 0.91$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$ + D_2O): δ 7.38–7.26 (Ar–*H*, m, 5H), 4.68 (C*H*–OH, d, $J = 3.35$ Hz, 1H), 3.19 (C*H*–NH_2, dt, $J = 6.38$, 3.38 Hz, 1H), 2.74 (βH, dd, $J = 12.7$, 6.6 Hz, 1H), 2.53 ($\beta' H$, dd, $J = 12.4$, 5.8 Hz, 1H), 2.63 (αCH_{2PYR}, m, 4H), 1.75 (βCH_{2PYR}, m, 4H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 142.5, 128.2, 127.2, 125.9, 75.1, 66.9, 61.9, 53.9, 52.7. IR (neat): 3362, 3296, 3086, 2926, 1451 cm^{-1}. HRMS $C_{13}H_{21}N_2O$ [M + H] calcd. 221.1654, found 221.1649.

(1*R*,2*R*)-(+)-1-Phenyl-2-decanoylamino-3-(*N*-morpholino)-1-propanol [D-*threo*-PDMP] (1b). Compound **10b** (192.4 mg, 0.814 mmol) was dissolved in pyridine (dried over sieves) and was sequentially treated with *p*-nitrophenyl decanoate (239 mg, 0.814 mmol) and 1-hydroxybenzotriazole (10 mol %, 12.5 mg, 0.0814 mmol). Upon completion judged by TLC, the solvent was removed, and the residue was dissolved in CH_2Cl_2 and extracted with NaOH (5 × 20 mL, 1 M). The crude was chromatographed (4:1 CH_2Cl_2:MeOH, $R_f = 0.42$) to give 280 mg of **1b** as a light brown oil in 88% yield. $[\alpha]^{25}_D = 8.05°$ ($c = 0.3$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.36–7.25 (Ar–*H*, m, 5H), 5.89 (N*H*, bd, $J = 6.75$ Hz, 1H), 4.96 (C*H*–OH, d, $J = 3.73$ Hz, 1H), 4.29 (C*H*–NH, ddd, $J = 6.30$, 6.9 Hz, 1H), 3.73 (CH_2–O_{MOR}, t, $J = 4.53$ Hz, 4H), 2.64 (βH, dd, $J = 6.63$, 13.05 Hz, 1H), 2.58 (CH_2–N_{MOR}, t, $J = 5.53$ Hz, 4H), 2.51 ($\beta' H$, dd, $J = 6.63$, 13.05 Hz, 1H), 2.10 (αCH_2, t, $J = 7.52$ Hz, 2H), 1.49 (βCH_2, p, $J = 6.90$ Hz, 2H), 1.23 [$(CH_2)_6$, m, 12H], 0.87 (CH_3, t, 6.61 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.6, 140.9, 128.2, 127.5, 125.9, 75.0, 66.8, 59.6, 54.2, 51.1, 36.6, 31.8, 29.3, 29.2, 29.0, 25.6, 22.6, 14.0. IR (neat): 3428, 3017, 2956, 1656, 1509 cm^{-1}. MS (FAB) [M + H] for $C_{23}H_{39}O_3N_2$; HRMS calcd. 391.2961, found 391.2980.

D-*threo*-PDMP (150 mg, 0.384 mmol) was dissolved in MeOH (5.0 mL), cooled to 0 °C, and acidified to pH 4.0 with HCl (3 M). The resulting mixture was concentrated in vacuo to afford a white crystalline compound. The crystalline product was recrystallized ($CHCl_3$:Et_2O) to give D-*threo*-PDMP·HCl·H_2O (126.5 mg, 0.296 mmol) in 77% yield. MP: 94–96 °C; $[\alpha]^{25}_D = -12.1°$ ($c = 0.40$, $CHCl_3$). IR (CH_2Cl_2): 3442, 2932, 1635 cm^{-1}. Elemental analysis (C, H, N): $C_{23}H_{38}N_2O_3$·HCl·H_2O calcd. 62.08% C, 9.29% H, and 6.30% N; found 61.76% C, 9.19% H, and 6.25% N.

(1*R*,2*R*)-(+)-1-Phenyl-2-decanoylamino-3-(*N*-thiomorpholino)-1-propanol [D-*threo*-PDTMP] (1c). Compound **10c** (81.4 mg, 0.323 mmol) was dissolved in pyridine (dried over sieves) and was sequentially treated with *p*-nitrophenyl decanoate (95 mg, 0.322 mmol) and 1-hydroxybenzotriazole (10 mol %, 5.0 mg, 0.032 mmol). Upon completion judged by TLC, the solvent was removed, and the residue was dissolved in CH_2Cl_2 and extracted with NaOH (5 × 20 mL, 1 M). The crude was chromatographed (20:1 CH_2Cl_2:MeOH, $R_f = 0.48$) to give 90 mg of **1b** as a light brown oil in 81.3% yield. $[\alpha]^{25}_D = +2.83°$ ($c = 1.17$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 7.36–7.25 (Ar–*H*, m, 5H), 5.82 (N*H*, d, $J = 7.03$ Hz, 1H), 4.93 (C*H*–OH, d, $J = 3.69$ Hz, 1H), 4.25 (C*H*–NH, ddd, $J = 10.77$, 6.63, 3.94 Hz, 1H), 2.82 (CH_2–N_{TM}, m, 4H), 2.69 (CH_2–S_{TM}, t, $J = 5.18$ Hz, 4H), 2.59 (βH, dd, $J = 13.17$, 6.60 Hz, 1H), 2.48 ($\beta' H$, dd, $J = 13.15$, 5.59 Hz, 1H), 2.09 (αCH_2, t, $J = 7.44$ Hz, 2H), 1.50 (βCH_2, p, $J = 7.51$ Hz, 2H), 1.24 [$(CH_2)_6$, m, 12H], 0.88 (CH_3, t, $J = 6.82$ Hz, 3H). ^{13}C NMR: (62.5 MHz, $CDCl_3$): δ 173.6, 140.9, 128.3, 127.5, 126.0, 75.1, 59.8, 55.7, 51.2, 36.7, 31.8, 29.3, 29.2, 29.18, 29.04, 27.9, 25.6, 22.6, 14.0. IR (neat): 3309, 2952, 1642, 1534 cm^{-1}. MS (FAB) [M + H] for $C_{23}H_{39}N_2O_2S$; HRMS calcd. 407.2732, found 407.2724.

(1*R*,2*R*)-(+)-1-Phenyl-2-decanoylamino-3-(*N*-piperidino)-1-propanol [D-*threo*-PDPiP] (1d). Compound **10d** (144.5 mg, 0.617 mmol) was dissolved in pyridine (dried over sieves) and was sequentially treated with *p*-nitrophenyl decanoate (181 mg, 0.617 mmol) and 1-hydroxybenzotriazole (10 mol %, 10 mg, 0.062 mmol). Upon completion judged by TLC, the solvent was removed, and the residue was dissolved in CH_2Cl_2 and extracted with NaOH (5 × 20 mL, 1 M). The crude was chromatographed (9:1 CH_2Cl_2:MeOH, $R_f = 0.58$) to give 164 mg of **1b** as a light brown oil in 68.8% yield. $[\alpha]^{25}_D = +6.33°$ ($c = 0.215$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 7.37–7.22 (Ar–*H*, m, 5H), 5.91 (N*H*, d, $J = 7.19$ Hz, 1H), 4.96 (C*H*–OH, d, $J = 3.56$ Hz, 1H), 4.31 (C*H*–NH, dt, $J = 11.25$ Hz, 1H), 2.62 (βH, dd, $J = 13.24$, 6.37 Hz, 1H), 2.50 ($\beta' H$, dd, $J = 13.49$, 5.26 Hz, 1H), 2.54 (αCH_{2PIP}, m, 4H), 2.08 (αCH_2, t, $J = 7.28$ Hz, 2H), 1.63 (βCH_{2PIP}, bp, $J = 5.28$ Hz, 4H), 1.49 (γCH_{2PIP}, m, 4H), 1.23 [$(CH_2)_6$, m, 12H], 0.87 (CH_3, t, $J = 6.38$ Hz, 3H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 173.4, 140.9, 128.1, 127.3, 126.0, 75.5, 59.9, 55.4, 50.6, 36.6, 31.7, 29.3, 29.2, 29.16, 28.9, 25.9, 25.5, 23.7, 22.5, 14.0. IR (neat): 3308, 2926, 1644, 1537 cm^{-1}. MS (FAB) [M + H] for $C_{24}H_{41}O_2N_2$; HRMS calcd. 389.3168, found 389.3170.

(1*R*,2*R*)-(+)-1-Phenyl-2-decanoylamino-3-(*N*-pyrrolidino)-1-propanol [D-*threo*-PDPP] (1e). Compound **10b** (33 mg, 0.015 mmol) was dissolved in pyridine (dried over sieves) and was sequentially treated with *p*-nitrophenyl decanoate (43 mg, 0.015 mmol) and 1-hydroxybenzotriazole (10 mol %, 2.5 mg, 0.0015 mmol). Upon completion judged by TLC, the solvent was removed, and the residue was dissolved in CH_2Cl_2 and extracted with NaOH (5 × 20 mL, 1 M). The crude was chromatographed (9:1 CH_2Cl_2:MeOH, $R_f = 0.23$) to give 41 mg of **1b** as a light brown oil in 74.2% yield. $[\alpha]^{25}_D = +2.26°$ ($c = 0.96$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 7.29–7.17 (Ar–*H*, m, 5H), 5.84 (N*H*, d, $J = 7.05$ Hz, 1H), 4.99 (C*H*–OH, d, $J = 3.2$ Hz, 1H), 4.19 (C*H*–NH, ddd, 8.04, 4.98, 4.92 Hz, 1H), 2.81 (βCH_2, d, $J = 5.12$ Hz, 2H), 2.68 (αCH_{2PYR}, m, 2H), 2.64 (βCH_{2PYR}, m, 2H), 1.99 ($\alpha' CH_2$, m, $J = 11.63$, 4.63 Hz, 2H), 1.75 (m, 4H), 1.40 ($\beta' CH_2$, p, $J = 7.29$ Hz, 2H), 1.27–1.09

[$(CH_2)_6$, m, 12H], 0.812 (CH_3, t, J = 7.12 Hz, 3H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 173.5, 140.9, 128.2, 127.4, 125.8, 75.3, 57.7, 55.2, 52.3, 36.7, 31.8, 29.7, 29.4, 29.3, 29.0, 25.6, 23.6, 22.6, 14.0. IR (neat): 3352, 2924, 1645, 1536 cm^{-1}. MS (FAB) [M + H] for $C_{23}H_{39}N_2O_2$; HRMS calcd. 375.3012, found 375.3005.

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Supporting Information Available: ^1H and ^{13}C data for compounds **1b–e**, **4b**, **5b**, **8b**, **9b–e**, and **10b–e** (29 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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